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Short communication

Chiral separation of racemic mexiletine hydrochloride using cyclodextrins as chiral additive by capillary electrophoresis

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Abstract

Enantiomeric separation of racemic mexiletine hydrochloride was performed using cyclodextrins (CDs) including α-CD, β-CD, heptakis-2,6-di-O-methyl-β-CD (DM-β-CD), heptakis-2,3,6-tri-O-methyl-β-CD (TM-β-CD), 2-O-(2-hydroxypropyl)-β-CD and γ-CD as chiral selectors. However, the enantiomers can be chirally separated only by methyl-β-CDs. The enantiomers could be baseline separated when TM-β-CD was used, and were only partially separated by DM-β-CD and no chiral separation was obtained using β-CD; thus, it is believed that methoxy groups of methyl-β-CDs play a key role in the chiral recognition for racemic mexiletine. Effects of CD concentration, applied voltage and the organic additive on chiral separation were studied. Under the conditions of 40 mmol/l Tris–H_3PO_4 buffer at pH 2.5 containing 20 mmol/l TM-β-CD, baseline separation (R_s=2.3) of the enantiomers can be achieved. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Buffer composition; Pharmaceutical analysis; Mexiletine hydrochloride

1. Introduction

During the past several years, it has been demonstrated that capillary electrophoresis (CE) is a powerful technique for chiral separation [1]. A number of pharmaceutical drugs and amino derivatives have been chirally resolved employing various chiral additives, such as cyclodextrins (CDs) and their derivatives [2–10], crown ether [11,12], dextrins [13], chiral surfactants [14,15], proteins [16–18], optical metal chelate complexes [19] and recently, macrocyclic antibiotics [20–22]. In most cases, CDs were used as chiral additives in CE in the background electrolyte [1].

Mexiletine hydrochloride is an antiarrhythmic drug used clinically as a racemic mixture. It is known that the enantiomers differ in their pharmaceutal effect [23,24]. The enantiomers of mexiletine have been separated as diastereomeric derivatives by achiral high-performance liquid chromatography (HPLC) [25–27] and gas chromatography (GC) [28]. The direct chiral separation of enantiomers of mexiletine had also been accomplished by HPLC [29] and GC [30].

In this paper the chiral separation of mexiletine hydrochloride was performed by CE with CD as chiral selector. The separation was systematically optimised. When heptakis-2,3,6-tri-O-methyl-β-CD (TM-β-CD) was used as chiral selector, baseline separation was achieved for mexiletine enantiomers under the conditions of 40 mmol/l Tris–H_3PO_4

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buffer (pH 2.5) containing 20 mmol/l TM-β-CD and applied voltage of 18 kV.

2. Experimental

2.1. Instrumentation

All the experiments were carried out on a BioFocus 3000 CE system (Bio-Rad, USA). The separation capillary was fused-silica with an internal diameter of 50 μm, a total length of 50 cm and an effective length of 45 cm. Before use, the capillary was rinsed with 0.1 mol/l NaOH solution for 10 min, and then with deionized water and running buffer for 4 min each.

2.2. Reagent and materials

β-CD was obtained from Yunan Cyclodextrin Factory (Guangdong province, China), it was recrystallized before use; α-CD and γ-CD were purchased from Sigma (St. Louis, MO, USA). Heptakis-2,6-di-O-methyl-β-CD (DM-β-CD) and TM-β-CD were synthesised according to the literature [31]; the average degree of substitution for DM-β-CD is 1.8 and for TM-β-CD is 2.6 (calculated from the data of elemental analysis). 2-O-(2-Hydroxypropyl)-β-CD (HP-β-CD) was synthesised according to the literature [32]. Tris(hydroxymethylaminomethane), phosphoric acid and methanol were purchased in China, racemic mexiletine hydrochloride was obtained from the Chinese Institute for Control of Pharmaceutical and Biological Products (Beijing, China); its chemical structure is shown in Fig. 1. In all experiments, deionized water was used.

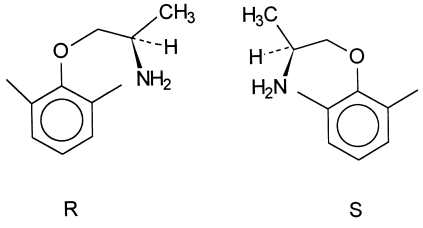

Fig. 1. Chemical structure of mexilitine hydrochloride enantiomers.

2.3. Electrophoresis

Mexiletine hydrochloride was dissolved in deionized water at a concentration of 0.1 mg/ml. The running buffer was composed of 40 mmol/l Tris and the buffer pH was adjusted to pH 2.5 by phosphoric acid. The various amounts of CDs were dissolved in this Tris–phosphate buffer. The separation capillary was thermostated at 20°C. The sample was pressure injected by 4 p.s.i.·s (1 p.s.i.=6894.76 Pa) and detected at 210 nm. Every two runs, the capillary was rinsed with running buffer for 4 min.

3. Result and discussion

3.1. The effect of CD type and CD concentration on chiral separation

In this experiment, six CDs: α-CD, β-CD, γ-CD, HP-β-CD, DM-β-CD and TM-β-CD were used as chiral selector for separation of mexiletine enantiomers. However, the enantiomers can be separated only by methylated β-CDs (DM-β-CD and TM-β-CD), and the chiral selectivity of TM-β-CD is better than that of DM-β-CD. It seems that the chemical modification of 2- and 3-hydroxy groups of β-CD with methoxy improved the chiral recognition of β-CD for mexiletine enantiomers. The probable explanation is that methylation of the hydroxy groups at 2, 3 positions of CD enlarged the rim of CD and makes the hydrophobic cavity flexible; thus the conformation of methylated β-CD (especially TM-β-CD) is better fitted to mexiletine enantiomers for the chiral recognition interaction. It is believed that α-CD and γ-CD have only weak interactions with the mexiletine molecule, because the effect of α-CD or γ-CD concentration on the mobility of mexiletine is weak. This may be due to the cavity of α-CD being too small and that of γ-CD too large for the mexiletine molecule to form stable inclusion complexes.

The effect of concentrations of DM-β-CD and TM-β-CD on chiral separation was investigated. Fig. 2 shows the dependence of the apparent mobility difference of enantiomer pairs on DM-β-CD or TM-β-CD concentrations. For DM-β-CD, it can be seen

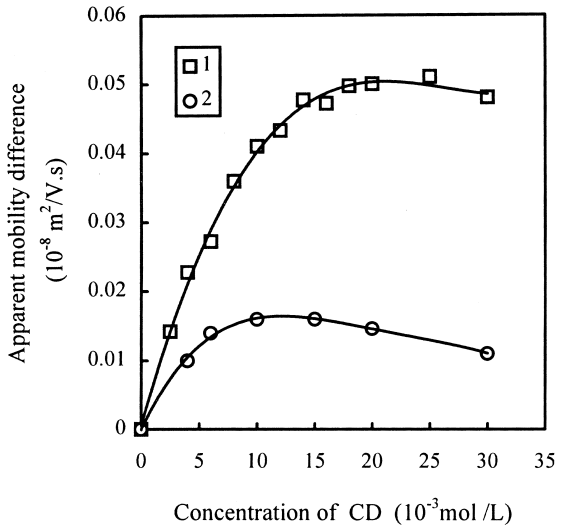

Fig. 2. Dependence of apparent mobility difference on CD concentration. Conditions: capillary, 50 μm I.D.×350 μm O.D. with 50 cm total length and 45 cm effective length; background electrolyte, 40 mmol/l Tris–H_3PO_4 buffer at pH 2.5; applied voltage, 18 kV; 1=TM-β-CD, 2=DM-β-CD.

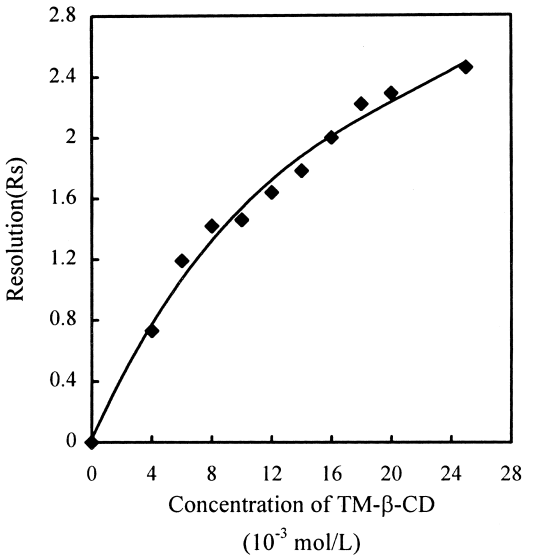

Fig. 3. Dependence of the resolution on TM-β-CD concentration. Conditions are the same as in Fig. 2.

that the apparent mobility difference reached a maximum value at a concentration of about 12 mmol/l, then decreased with increasing DM-β-CD concentration. Under the optimum concentration of DM-β-CD, the enantiomers were only partially separated. The improved separation could be obtained when TM-β-CD was used as chiral additive. Also in Fig. 2, it can be seen that the apparent mobility difference increased as the concentration of TM-β-CD increased; the maximum value appeared at a concentration of about 25 mmol/l. Comparing the two curves in Fig. 2, the trends appear to be different. The optimum concentration of DM-β-CD appeared early than that of TM-β-CD, but the chiral selectivity of TM-β-CD for mexiletine enantiomers is obvious better than that of DM-β-CD. According to the chiral separation model of CE presented by Wren and Rowe [3], the curves of different types implied different interaction between CD and chiral compound.

The dependence of resolution (R_s) on TM-β-CD concentration is shown in Fig. 3. The R_s between two enantiomers was calculated by the equation: $R_s = 1.18\frac{(t_2 - t_1)}{(w_{h1} + w_{h2})}$, where t_1 and t_2 are the migration times of two enantiomers, w_{h1} and w_{h2} are the peak widths at the half height of the peak. Fig. 4 shows the chiral separation of mexiletine enantiomers. It

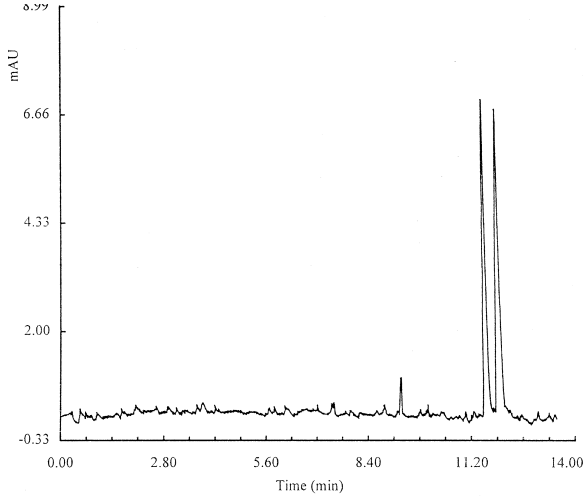

Fig. 4. Electropherogram for the chiral separation of mexiletine enantiomers. Conditions: capillary, 50 μm I.D.×350 μm O.D. with 50 cm total length and 45 cm effective length; background electrolyte, 40 mmol/l Tris–H_3PO_4 buffer at pH 2.5 containing 20 mmol/l TM-β-CD; applied voltage, 18 kV; current, 14 μA.

can be seen that the enantiomers were baseline separated by TM-β-CD.

3.2. Effect of organic additive on chiral separation

Methanol was added at different concentrations to the running buffer in an attempt to improve the chiral selectivity of DM-β-CD; however, the chiral selectivity decreased with rising concentrations of methanol. When the methanol concentration reached 20% (v/v), DM-β-CD lost the chiral recognition for mexiletine enantiomers.

3.3. Effect of the applied voltage on chiral separation

The effects of the applied voltage on chiral separation parameters are shown in Fig. 5. It can be seen that R_s decreased when the voltage rose from 15 kV to 24 kV. This could be explained by the following: as the applied voltage increases, the increasing Joule heat leads to a reduced separation efficiency; on the other hand, the apparent mobility increased with increasing voltage, and the relative migration time of enantiomeric pairs decreased.

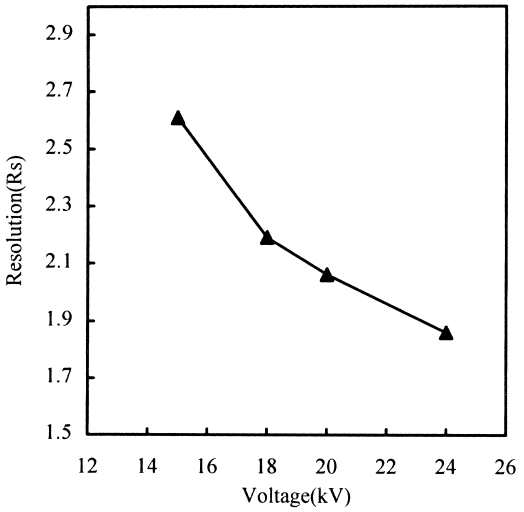

Fig. 5. Effect of the applied voltage on chiral separation. Conditions: capillary, 50 μm I.D.×350 μm O.D. with 50 cm total length and 45 cm effective length; background electrolyte, 40 mmol/l Tris–H_3PO_4 buffer at pH 2.5 containing 20 mmol/l TM-β-CD. Currents: 13 μA (15 kV), 14 μA (18 kV), 19 μA (20 kV) and 23 μA (24 kV).

4. Conclusions

A CE method for the chiral separation of mexiletine hydrochloride enantiomers is reported. Among the six CDs employed as chiral additives, only methylated β-CDs showed chiral recognition for mexiletine hydrochloride enantiomers, and TM-β-CD offered better chiral selectivity than DM-β-CD. Under the optimum conditions of 40 mmol/l Tris–phosphate buffer (pH 2.5) containing 20 mmol/l TM-β-CD and the applied voltage of 18 kV, the enantiomers could be baseline separated.

Acknowledgements

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